A risk factor analysis of outcomes after unrelated cord blood transplantation for children with Wiskott-Aldrich syndrome

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Abstract

Wiskott-Aldrich syndrome is a severe X-linked recessive immune deficiency disorder. A scoring system of Wiskott-Aldrich syndrome severity (0.5-5) distinguishes 2 phenotypes: X-linked thrombocytopenia and classic Wiskott-Aldrich syndrome. Hematopoietic cell transplantation is curative for Wiskott-Aldrich syndrome, however the use of unrelated umbilical cord blood transplantation has seldom been described. We analyzed umbilical cord blood transplantation outcomes for 90 patients. Median age at umbilical cord blood transplantation was 1.5 years. Patients were classified according to clinical scores (2 (23%), 3 (30%), 4 (23%) and 5 (19%)). Most patients received HLA mismatched umbilical cord blood transplantation and myeloablative conditioning with anti-thymocyte globulin. Cumulative incidence of neutrophil recovery at day-60 was 89% and day-100 acute graft-versus-host disease grade II-IV was 38%; use of methotrexate for graft-versus- host disease prophylaxis delayed engraftment (p=0.02), but decreased acute graft-versus-host disease (p=0.03). At 5-year, overall survival and event-free survival were 75% and 70%, respectively. Estimated 5 year- event-free survival was 83%, 73% and 55% for patients with clinical score 2, 4-5 and 3, respectively. In multivariate analysis, age<2years at umbilical cord blood transplantation and clinical phenotype X-linked thrombocytopenia were associated with improved event-free survival. Overall survival tended to be improved after 2007 (p=0.09). In conclusion, umbilical cord blood transplantation is a good alternative option for young children with Wiskott-Aldrich syndrome lacking an HLA identical stem cell donor.

Introduction

Wiskott-Aldrich syndrome (WAS) is a severe X-linked recessive immune deficiency disorder caused by mutations in the gene encoding for Wiskott-Aldrich syndrome protein (WASP), a key regulator of actin polymerization signaling and cytoskeletal reorganization in hematopoietic cells[1-3]. A mutation in *WASP* results in a wide spectrum of clinical manifestations ranging from the relatively mild X-linked thrombocytopenia (XLT) to the classic WAS phenotype characterized by microthrombocytopenia, immunodeficiency, eczema, and high susceptibility to lymphoproliferative tumors and autoimmune diseases. [2,4,5] A simple scoring system on a scale of 0.5 to 5 was introduced to differentiate XLT from classic WAS patients based on the severity of the clinical phenotype (supplemental table 1). [6]

XLT patients (score <3) have excellent overall survival (OS), but have, also, a high probability of severe disease-related complications[7]. In contrast, the classic WAS (score ≥3) usually leads to death in early childhood or adolescence, despite advances in clinical care, with median life expectancy of only 15 years. [6,8,9]

Currently, the only proven curative therapy for patients with WAS is hematopoietic stem cell transplantation (HSCT).[6,10] Various series of HSCT from HLA-matched related donors (MRDs) have consistently resulted in survival rates above 80% for patients with WAS [11-15]. In the absence of a MRD, the OS reported after matched unrelated HSCT has been around 70%[11]. In the last 20 years, unrelated donor umbilical cord blood transplantation (UCBT) has become an option for patients lacking a HLA matched donor[16]. To date, there are only few reports on outcomes after UCBT for patients with primary immune deficiencies, [17-19] but they include only a few patients with WAS, [18,20-22] and none of the studies have analyzed factors associated with outcomes after UCBT. Therefore, we conducted a collaborative multi-center retrospective risk factor analysis of patients with WAS reported to Eurocord. A total of 90 UCBT recipients met the criteria and were included in the study.

Methods

Data collection

This retrospective analysis is based on data reported to EBMT and/or Eurocord from European and non-European transplant centers through a standardized questionnaire that included information on patients, donors, diseases, and transplant outcomes. Missing information was requested in the form of a Microsoft Excel file listing transplantations performed by each centre along with key data extracted from the Eurocord-EBMT databases. In addition, data from Duke University Medical Center (USA), Federal University of Parana (Brazil) and Pontifical Catholic University of Chile were obtained from the respective center. Recipient's parents or legal guardians gave informed consent for HSCT according to the Declaration of Helsinki. Eurocord and the Working Party of Inborn Errors of EBMT approved this study.

Inclusion criteria

The inclusion criteria for the study were: (1) Patients transplanted for WAS before December 31, 2013, and reported to Eurocord; (2) First allogeneic unrelated HSCT.
Patients were excluded from the study if diagnosis of immune deficiency was not specified or if transplants were performed with a cord blood unit that was expanded, combined with other HSC source, or injected intra-bone.

Endpoints and definitions

The primary endpoint was: event-free survival (EFS), defined as survival from transplantation to last contact without any of the following events: autologous reconstitution (defined by documentation of <5% donor derived engraftment), graft failure (defined as a lack of neutrophil recovery or transient engraftment of donor cells after transplantation, and/or a requirement for a second transplant) and death. All surviving patients were censored at the date of last contact.

Other endpoints reported included: (1) overall survival (OS), defined as time from transplantation to death of any cause; (2) Cumulative incidence of neutrophil engraftment, defined as the first day of achieving a neutrophil count of $\geq 0.5 \times 10^9$/L for 3

consecutive days with evidence of donor hematopoiesis; (3) Cumulative incidence of platelet engraftment defined as the first of 3 consecutive days post-HSCT with a platelet count ≥20x10^9/L without platelet transfusions for at least seven days; (4) Graft failure: primary failure defined as neutrophil never reaching 0.5x10^9/L or evidence of autologous reconstitution; secondary graft failure defined as reaching a neutrophil count of 0.5x10^9/L after transplant, but experiencing a subsequent, non-transitory, decrease, or loss of donor chimerism; and (5) Incidence of acute- and chronic graft versus host disease (GvHD; acute-GvHD grade II-IV was diagnosed and graded according to published criteria.[23] Chronic-GvHD was graded according to standard criteria[24] and evaluated in patients who survived at least 100 days with sustained engraftment.

Myeloablative conditioning regimen was defined as conditioning including an intravenous busulfan (IV Bu) total dose of more than 6.4 mg/kg or an oral dose greater than 8 mg/kg/day, or treosulfan (Treo) >36 mg/m^2 for infants (less than 12 months old) and >42 mg/m^2 for others. Other regimens were considered reduced intensity conditioning (RIC).

Donor-recipient HLA matching was defined considering low resolution typing for HLA Class I (A and B) and high resolution typing for HLA Class II (DRB1). Donor-recipient chimerism was reported on the basis of available data during the first 100 ±30 days, 1-year ±30 days after UCBT and at the last chimerism evaluation. Full-donor chimerism was defined as the presence of ≥95% donor-derived hematopoietic cells, mixed-chimerism as 5% to 94% of these cells and autologous recovery if <5%.

Immune phenotyping results ($CD3^+CD4^+$ and $CD3^+CD8^+$ T-lymphocytes; $CD19^+$ B lymphocytes) were measured at 100 ±30 days, 1-year ±30 days and at the last assessment reported after UCBT. As normal values in childhood vary considerably with age, absolute numbers of CD4+, CD8+ and CD19 were related to age-specific normal values.[25,26] Immune recovery was defined as being alive with neutrophil engraftment and achieving absolute number of CD4+, CD8+ and CD19+ within the age-related normal values as shown in a supplemental table 2.

Statistical analysis

To analyze risk factors for outcomes, we considered factors related to patient (median age at diagnosis, median age at transplant, median weight at time of transplantation, gender, pre-transplant CMV serology status, Lansky score), disease (pre-transplant information on: infections, severe thrombocytopenia (platelets<20x10^9/L), number of platelets transfusion, platelet abnormalities, history of severe bleeding, splenectomy, presence of eczema, autoimmunity, malignancy, congenital neutropenia, clinical phenotype, median interval from date of birth to diagnosis, median interval time from diagnosis to transplant)], cord blood unit (HLA matching, median collected and infused total nucleated cell and CD34+cell doses), and factors related to transplantation (year of transplant, use of RIC or MAC, type of GvHD prophylaxis).

Cumulative incidence curves were calculated for neutrophil, platelet engraftment, acute and chronic GvHD in a competing risk setting, with death as a competing event [27]. Gray test was used for univariate comparisons. Probabilities of EFS and OS were calculated using the Kaplan-Meier estimate; the two-sided log-rank test was used for univariate comparisons. Multivariate analysis were performed using Cox proportional hazard regression model [28] for EFS and OS, and proportional sub distribution hazard regression model of Fine and Gray for acute and chronic GvHD, neutrophil and platelets engraftment. Variables, which reached a p-value 0.10 in the univariate analysis, and other relevant factors such as HLA matching and cell dose, were included in the initial models and variables were eliminated one by one at a time in a stepwise fashion in order to only keep variables that reached a p-value of 0.05 in the final model. P-values were two-sided. Statistical analyses were performed using SPSS (Inc., Chicago) and S-Plus (MathSoft, Inc., Seattle) software packages.

Results

Patient, donor, and transplant characteristics

Ninety patients with a clinical diagnosis of WAS who received UCBT between 1996 and 2013 in 33 centers from 20 countries met the eligibility criteria for the study. The baseline patient, donor, and transplantation characteristics are shown in table 1. Disease severity before transplantation was expressed as a WAS score of 2 to 5. Eighteen patients (23%) had a WAS clinical score of less than 3 at the time of UCBT, indicating that they had not experienced any of the following: severe infections, difficult-to-treat

eczema, autoimmunity, or malignancy. The majority of patients (n= 61, 77%) had severe clinical features of the disease at the time of transplantation, including 52 patients with a history of recurrent and/or severe infections. Seven patients had a history of autoimmune disorders and two of EBV associated lymphoproliferative disease.

Four patients were splenectomized before UCBT, of whom two had a platelet count $<20x10^9$cells/L at the time of transplantation. All of the splenectomized patients had the classic WAS clinical phenotype and three experienced severe infection before UCBT.

Data on *WAS* gene mutations were available for 39 patients (43%). Almost an equal proportion of patients carried nonsense, missense and deletion mutations.

The median age at transplant was 1.48 years (range 4.8 months – 14.25 years). Only 9 patients were more than 5 years old at the time of UCBT. Most patients (76%) had a good performance status at the time of UCBT (Lansky score >80%).

The vast majority of children were conditioned with a busulfan containing myeloablative regimen (97%), mainly busulfan/cyclophosphamide (76%). Three patients received reduced-intensity conditioning, two of them due to severe infection at time of UCBT. Most patients received anti-thymocyte globulin (ATG) (n=79). All patients who received GvHD prophylaxis received a calcineurin inhibitor containing regimen, consisting of either CsA (in most of cases) or Tacrolimus. The median number of total nucleated cell and CD34+ cell doses infused were 6.8 x 10^7 cells/kg and 3.04 x 10^5/kg (pre-cryopreservation counts), respectively.

Neutrophil and platelets recovery

Eighty patients (89%) achieved neutrophil engraftment, with a median time to engraftment of 21 days (range, 9-54). The cumulative incidence (CI) of neutrophil engraftment was 70%, and 89% at day 30 and 60, respectively. Ten (11%) patients did not achieve neutrophil engraftment. Of the patients who failed to engraft, 4 received a second transplantation and were alive at last follow-up, and the 6 who did not receive a second HSCT, died.

Multivariate analysis showed that use of methotrexate (MTX) in GvHD prophylaxis (HR 0.55, 95% CI 0.32-0.93, p=*0.02*) was associated with lower CI of neutrophil engraftment.

At day 180, CI of platelet engraftment was 75%, with a median time to engraftment of 45 days (range, 11-224). Platelet engraftment in the 4 splenectomized patients seemed

faster with median time of 35 days. Due to small number of splenectomized patients, it was not possible to confirm if there was a real impact of this procedure in the engraftment rate. In univariate analysis, the only risk factor associated with lower CI of platelet engraftment was age more than 2 years (67% vs. 79% for younger patients, p= 0.03). Multivariate analysis confirmed that age was independently associated with platelets engraftment (HR 0.34, 95% CI 0.16-0.73, *p=0.005*).

Chimerism and immune recovery

Chimerism data was available for 66 (86%) out of 77 evaluable patients at 100 (±30) days post-UCBT, 50 (80%) out of 63 patients at 1-year (±30 days) post-UCBT, and 51 (82%) out of 62 patients at last assessment. At day 100, 68% of patients were full donor and 32% mixed chimerism; at 1 year, 76% and 24%, respectively and at last assessment, 80% and 20%, respectively. In 12 cases, mixed chimera became full donor in a further assessment, and 2 patients who were, initially, full donor became stable mixed chimera at last assessment.

Information on the absolute number of $CD3/4^+$, $CD3/8^+$ and $CD19^+$ lymphocytes at 100±30 days, 1 year±30 days after UCBT and at the latest assessment was available in 29, 25 and 25 of the patients who were alive with neutrophil engraftment at specific time-points, respectively. In this subset analysis, 31 (67%) out of 46 patients achieved IR. Median time between UCBT and the first reported immune recovery testing was 12 months; eleven patients achieved IR before the first 12 months after transplantation, the earliest confirmed IR was reported 4 months after UCBT. Fifteen patients did not achieve IR. Of these, 13 patients were being treated with immunosuppressive agents due to acute GvHD, remaining two patients, who had no history of GvHD, had mixed chimerism results.

Acute and chronic GvHD

Acute GvHD grade II-IV was observed in 35 patients (22 - experienced grade II (24%), 8 - grade III (8%), and 5 - grade IV (6%)). The CI of acute GvHD II-IV at day 100 was 38%. In univariate analysis, none of the factors analyzed were significantly associated with an increased risk of grade II-IV GvHD. However, in multivariate analysis, the use of MTX for GvHD prophylaxis was associated with decreased incidence of grade II-IV GvHD (22%

vs. 42%) (HR 0.34, CI 95% 0.12-0.91, p=*0.03*). CI of chronic GvHD at 5 years was 17% (n=15; 6 extensive and 9 limited cases). In univariate analysis, cumulative incidence of chronic GvHD decreased after 2007 (25% vs. 3%, *p<0.01*). Chronic GvHD also decreased for patients receiving a TNC cell dose lower than 6.8 x10^7/kg, (23% vs. 7%, *p=0.03*). In multivariate analysis, none of the risk factors studied were significantly associated with an increased risk of chronic GvHD.

Overall survival, event-free survival and causes of death

The probability of 5-year overall survival (OS) and event-free survival (EFS) were 75±5% and 70±5%, respectively. Table 2 shows the univariate analysis of risk factors for OS and EFS. The risk factors associated with worst OS in multivariate analysis (table 3) were: age >2 years at UCBT [HR: 2.61, 95%CI: 1.1-6.16; p=0.02] and clinical score > 2 (XLT) [HR: 4.49; 95%CI: 1.02-19.78; p=0.04] (Figure 1-2). There was a trend of improved OS in patients transplanted after 2007 [HR 2.27, 0.86-5.98, p=0.09] (Figure 3). In multivariate analysis for EFS, older children (more than 2 year) at UCBT also had significantly worse prognosis [HR: 2.47, 95%CI: 1.1-5.52; p=0.02]. Sixty-seven patients were alive at last assessment, with a median follow-up of 5 years (range, 0.25-17). Twenty-three (25%) patients died. Table 4 shows causes of death before and after 100 days post UCBT, according to WAS score. Infection related deaths were commonly observed among all disease scores, and it was the main cause of death, especially before day 100.

Discussion

This multicenter, retrospective study on UCBT recipients with WAS confirms that for most patients, HSCT using HLA matched or mismatched CB cells can cure and prevent the long-term, life-threatening complications associated with WAS. Outcomes of patients with WAS who do not undergo HSCT remain poor, with the mean age of death of 20 years in previous reports[9] and with increasing risk of malignancies with age. Several groups[11,16, 15] reported successful HSCT results with an OS of up to 88% when using a "gold standard donor" [14]. In the absence of a matched related donor (MRD), other groups have reported the successful use of matched unrelated donors with 71% OS

[11,29,30], but with higher risk of acute and chronic GvHD. Unfortunately, many patients do not have an available matched unrelated donor. Therefore, other donor sources for transplantation have been investigated, such as the use of T-cell depleted haploidentical HSCT and umbilical cord blood HSCT. Nowadays, the results after haploidentical HSCT with TcRαβ/CD19-depletion for patients with PID seem promising[31]. On the other hand, UCB is still attractive due to the naivety of the stem cells, the lower HLA matching requirements, and easy availability (compared to MRD and MUD) and decreased GvHD (compared to haploidentical HSCT)[32].

We have conducted a risk factor analysis for this rare disease using retrospective-registry based data. The limitations of our study, is mainly due to some missing data related to the disease and the long inclusion period with changes in cord blood unit selection and better supportive care in most recent years. Despite these limitations, the study remains noteworthy, as it is the largest series of children with WAS treated with UCBT.

We were able to identify mainly 2 main factors associated with EFS and OS after UCBT: age at UCBT and clinical disease score (Figure 1-2). EFS and OS were significantly improved when transplantation was performed before 2 years of age with almost 80% of these young patients cured. This finding supports the need for early referral for transplantation in infants diagnosed with WAS. We could speculate that older children could have more previous complications before UCBT, which, in turn, could affect outcomes. However, in patients with available data, we determined that OS and EFS were not associated with number of previous infections, Lansky score, severity of eczema, thrombocytopenia, and number of previous platelets transfusions, autoimmunity and congenital neutropenia.

Clinical score was also a prognostic factor. As expected, patients with XLT (score <3) had improved OS and EFS when compared with other clinical phenotypes. XLT patients have, historically, excellent OS without transplantation in contrast to patients with classic WAS. However, EFS in XLT patients seem to worsen over time[33,34]. Data from the XLT registry showed EFS of 74% at 15 years, decreasing to 56% by 30 years, without subsequent transplantation. Currently, there is no consensus on indication of HSCT in XLT and decision on HSCT for such patients has to be made on individual basis[7]. In our cohort, 23% of the patients were classified as having score 2, and there were no

patient with score 0.5 or 1 (table 1), showing that some degree of severity was present to justify the transplantation. We found that patients with a clinical score of 3 seemed to have worse probabilities of OS and EFS, however due to small number of patients in the groups, we were not able to draw any definitive conclusions.

The most frequent cause of death after UCBT was infection. The majority of patients in our series received ATG as part of the conditioning regimen, which may explain the high number of infection related deaths observed. [35] Infections are commonly seen after UCBT due to delayed engraftment and impaired immune recovery mainly when ATG is used before UCBT[36].

We were able to analyze immune recovery in a subset of patients. We found that 67% of the 46 patients with available information achieved immune recovery. Median time between UCBT and first test reporting IR was 12 months, and eleven patients achieved IR before first 12 months after transplantation. These results seem comparable to the IR usually observed after UCBT. [26] However, due to the retrospective nature of our analysis, we were unable to collect data on IVIG use details and vaccine-specific antibody response; therefore the IR results reported here should be taken with caution. Most patients who did not achieve IR were treated with immune suppressive agents due to acute GvHD, which may explain our findings.

Mixed chimerism post-transplant is an undesirable outcome following HSCT for WAS since it may be associated with lymphopenia, autoimmunity and thrombocytopenia. [16] In our series, chimerism data was available for 86% of the patients during 3 months, 12 months and last assessment. Full donor chimerism was observed in 68%, 76% and 80% of the patients, respectively. In a recent study of chimerism in 194 HSCT recipients with WAS, 72.1% of patients achieved full and stable donor chimerism.[16] In this study was also shown that mixed chimerism impacts the myeloid compartment (16.5% of cases), followed by B cell compartment (7.4% of cases) and uncommonly the T-cell compartment (3.2% of cases). Unfortunately, in our series, we did not have data on lineage specific chimerism, however our results are comparable with the 72% full donor chimera seen with other HSC sources.

We were unable to identify cord blood donor related factors associated with outcomes. Previously, the Eurocord group has reported that for UCBT recipients with non-malignant disorders, a cell dose higher than $5x10^7$/kg and a 6/6 or 5/6 HLA grafts

are associated with decreased mortality[35]. In our study, patients were transplanted with a median cell dose of 7.5 x10^7/kg and 70% received a 6/6 or 5/6 CB graft, which is in agreement with the recommendations for cord blood selection for patients with non-malignant disorders.

In conclusion, early referral for UCBT in patients with WAS is associated with better outcomes. New treatment strategies such as autologous gene-modified HSCT may overcome the disadvantages of graft rejection and GvHD after allogeneic HSCT. However, until these strategies become clinically available, UCBT remains a good alternative for patients lacking an HLA matched donor.

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Authorship

Contributions: VR and EG designed the study; VR, AR and ZS, performed statistical analysis; ZS prepared data and wrote the manuscript; CB, SN, KP, AA, FCB, JSTC, PV, JJB,

KM, HB, TO, PJS, AC, JK and AG provided and reviewed clinical data. FV and FG participated in data collection and analysis. All authors edited and approved the manuscript.

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Tables

Table1. Baseline patient, disease, donor and transplantation characteristics.

	N =90 (%*)	Median (range)
Patient characteristics		
Gender (Male/Female)	88/2	
Weight, kg		10.25 (5-51.7)
Age at diagnosis, years		0.32 (0-8.3)
Time interval diagnosis- UCBT, years		1.05 (0.06-13.05)
Age at transplantation, years		1.48 (0.40-14.25)
≤5	81	
> 5	9	
CMV before UCBT		
Seropositive	47(70)	
Seronegative	20 (30)	
Disease characteristics		
WAS clinical phenotype at UCBT		
Severe infections	52 (68)	
Eczema		
Mild	19 (28)	
Moderate	35 (51)	
Severe	14 (21)	
Severe thrombocytopenia (<20x10^9cells/L)	43 (70)	
Microthrombocytopenia	22 (51)	
Life-threatening bleeding	41 (59)	
Malignancies	2 (4)	
Autoimmunity	7 (13)	
Congenital neutropenia	9 (19)	
WAS score		
2	18 (23)	
3	24 (30)	
4	18 (23)	
5	19 (24)	
Splenectomy before UCBT	4 (5)	
Donor characteristics		
HLA-matching		
6/6	11 (12)	
5/6	52 (58)	
4/6	25 (28)	
3/6	1	
Cell dose		
Collected NC (x10^7/kg)		7.5 (0.2-3)
Collected CD34+ (x10^5/kg)		3.03 (0.03-35)
Transplant characteristics		
Year of transplantation		2007 (1996-2013)
Conditioning regimen		
MAC	87 (97)	

Cy/Bu	67
Cy/Bu+others	8
Bu/Fluda	6
Fluda/Treo42	5
Cy/Hydroxyurea	1
RIC	3
Cy/Bu	1
Fluda/Melph ± Treo36	1/1
GvHD prophylaxis	
CNI/Pred	49 (54)
CNI/MTX	17 (20)
CNI/MMF	11 (12)
CNI	9 (10)
CNI/others	3 (3)
Non given	1 (1)
Serotherapy	
Anti-T serotherapy (before day 0)	79 (89)
Monoclonal Antibody	2 (2)
Not given	8 (9)

* Percentage of evaluable cases

UCBT indicates umbilical cord blood transplantation; CMV, cytomegalovirus; WAS, Wiskott-Aldrich syndrome; NC, nucleated cells; MAC, myeloablative conditioning regiment; Cy, cyclophosphamide; Bu, busulphan; Fluda, fludarabine; Treo, treosulphan; GvHD, "graft-versus-host" disease; CNI, calcineurin inhibitor; Pred, prednisolone.

Table 2. Probability of 5-year overall and event-free survival after UCBT for WAS

		5y OS % (95%CI)		5y EFS % (95%CI)	
Variable	N		*P*		*P*
Patients characteristics					
Age at UCBT, years					
≤ 2 years	60	83 (71-91)	*.027*	78 (67-86)	.05
> 2 years	30	58 (41-74)		55 (38-71)	
CMV status					
negative	19	77 (54-91)	*.9*	73 (51-88)	.9
positive	47	80 (66-89)		74 (59-85)	
Weight at UCBT, kg					
<10	45	89 (76-96)	*.002*	82 (68-91)	.02
≥ 10	45	60 (44-74)		58 (42-72)	
Splenectomy					
Yes	4	50 (15-85)	*0.2*	50 (15-85)	.33
No	75	76 (42-93)		72 (61-81)	
Lansky score, %					
<90	19	67 (44-84)	*.63*	57 (34-77)	.2
≥ 90	59	76 (63-86)		74 (61-84)	

Disease characteristics					
Clinical score					
2	18	89 (69-97)		83 (61-94)	
3	24	61 (41-77)	*.03*	55 (35-75)	.18
4	18	78 (55-91)		72 (50-88)	
5	19	79 (57-91)		74 (52-88)	
UCBT characteristics					
HLA-match					
6/6	11	71 (41-90)		61 (33-83)	
5/6	53	76 (63-86)	*.9*	72 (59-82)	.75
4/6 or 3/6	26	72 (52-86)		69 (50-83)	
ABO-compatibility					
no incompatibility	36	70 (47-86)		72 (55-850	
minor incompatibility	17	76 (58-88)	*.8*	70 (47-86)	.9
major incompatibility	29	70 (49-85)		70 (51-84)	
Year of UCBT					
Before 2007	51	69 (54-81)	*.13*	65 (51-77)	.2
After 2007	39	83 (66-93)		78 (62-51)	
Time from diagnosis to UCBT					
< 7 months	23	91 (73-97)		86 (68-95)	
7-13 months	22	80 (56-93)	*.14*	76 (53-90)	.18
>13-19 months	22	72 (50-87)		68 (47-83)	
> 19 months	22	58 (37-77)		54 (34-73)	
Conditioning regimen					
Cy/Bu/ATG	25	83 (64-93)	*.2*	83 (60-91)	.3
others	65	72 (60-81)		67 (55-77)	
GvHD prophylaxis					
MTX	18	74 (63-83)	*.7*	70 (57-80)	.7
others	72	78 (54-91)		72 (48-88)	
Cell doses					
TNC collected, $x10^7$/kg					
< 7	43	72 (57-83)	*.43*	67 (52-79)	.4
≥ 7	42	77 (61-88)		73 (58-84)	
TNC infused, $x10^6$/kg					
< 6	43	77 (61-88)	*.8*	74 (59-85)	.5
≥ 6	42	72 (57-83)		65 (49-79)	
CD34+collected, $x10^6$/kg					
< 3	34	68 (51-81)	*.2*	62 (46-76)	.12
≥ 3	33	78 (59-90)		75 (57-87)	
CD34+ infused, $x10^5$/kg					
Less than 3	34	66 (52-78)	*.11*	74 (59-85)	.14
At least 3	33	86 (64-95)		75 (57-87)	

UCBT indicates umbilical cord blood transplantation; WAS, Wiskott-Aldrich syndrome; OS, overall survival; EFS, event-free survival; CI, confidence interval; CMV, cytomegalovirus; Cy, cyclophosphamide; Bu, busulphan; ATG, anti-thymocyte globulin; GvHD, graft-versus-host disease; TNC, total nucleated cells.

Table 3. Multivariate analysis for OS and EFS

		HR	95% CI	*P*
OS	Older than 2 years	4.49	1.02-19.78	**.04**
	WAS score more than 2	2.61	1.1-6.16	**.02**
	UCBT after 2007	2.27	0.86-5.98	.09
EFS	Older than 2 years	2.47	1.1-5.52	**.02**
	WAS score more than 2	3.13	0.9-10.87	.07
	UCBT after 2007	1.68	0.7-4.04	.24

OS indicates overall survival; EFS, event-free survival; HR, Hazard ratio; CI, confidence interval; WAS, Wiskott-Aldrich syndrome; UCBT, umbilical cord blood transplantation.

Table 4. Primary causes of death before and after 100 days after UCBT for WAS

Primary causes of death	**WAS clinical score**					**Total number**
	2	3	4	5	Unknown	
N deaths/N total	2/18	11/24	4/18	4/19	2/11	
Before 100 d	**1**	**5**		**1**		**7**
Viral infection		3				3
Fungal infection	1			1		2
Bacterial infection		1				1
Unknown infection		1				1
After 100 d	**1**	**6**	**4**	**3**	**2**	**16**
Infection		5		1	1	7
GvHD	1			2		3
Multiorgan failure			2			2
Unknown			1		1	2
Interstitial pneumonitis			1			1
Hemorrhage		1				1

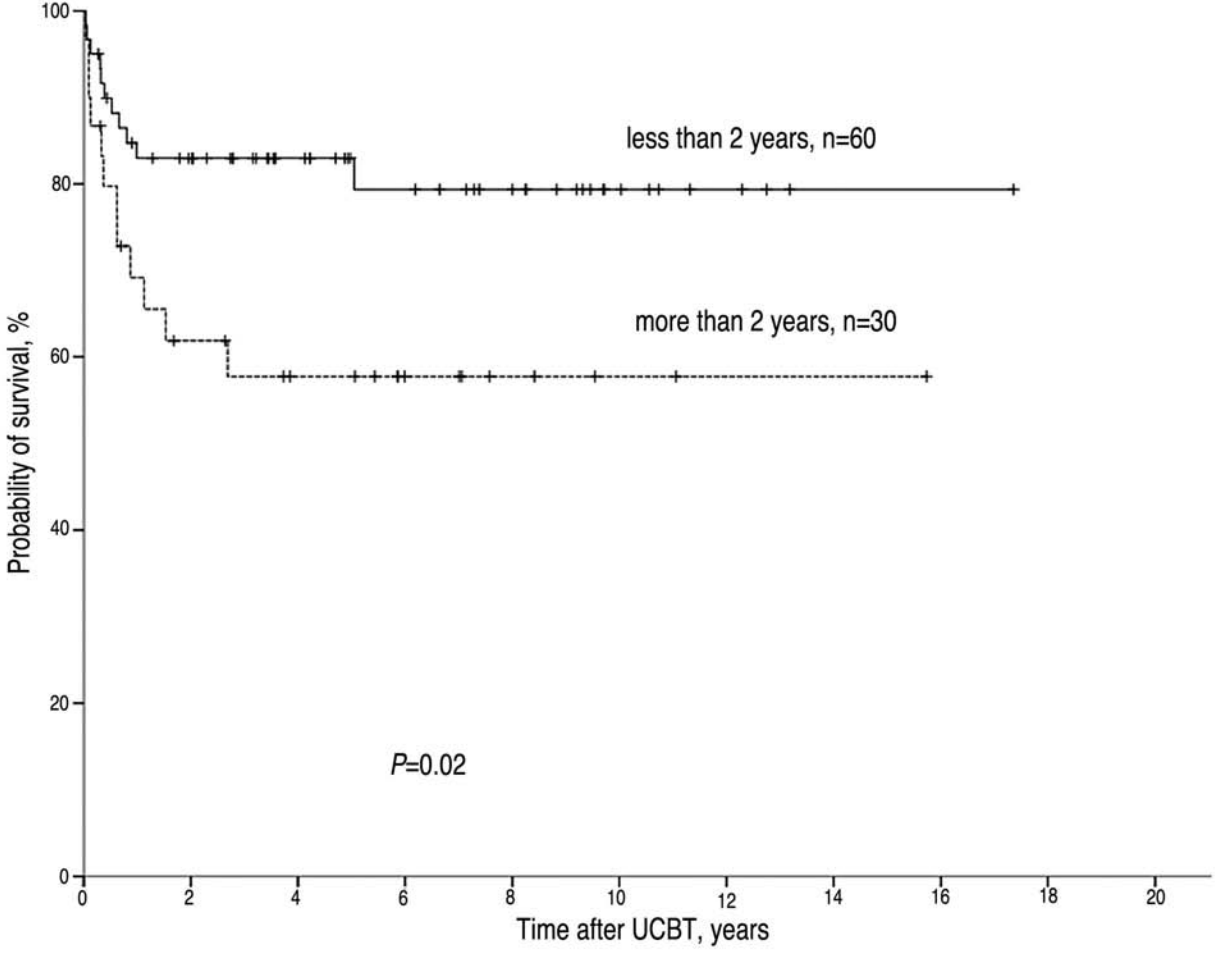
UCBT indicates umbilical cord blood transplantation; WAS, Wiskott-Aldrich syndrome; GvHD, "graft-versus-host" disease.

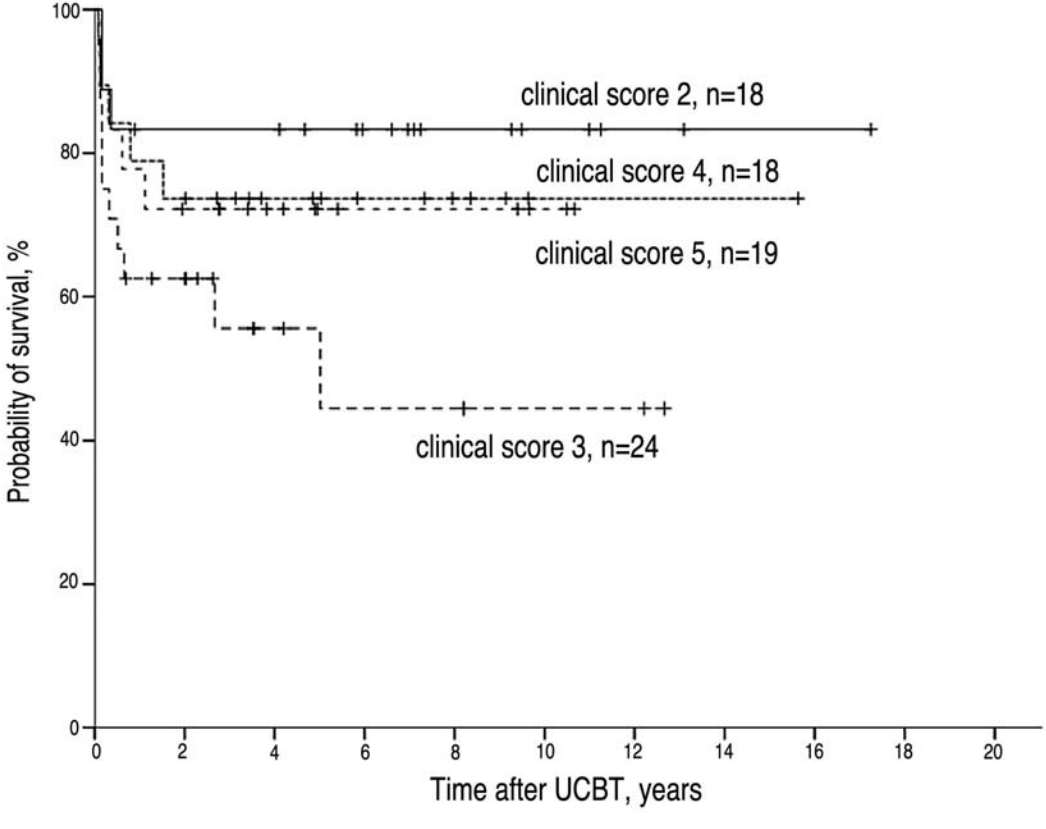
Figures legends

Figure 1. Probability of overall survival after umbilical cord blood transplantation for Wiskott-Aldrich syndrome according to age.

Figure 2. Probability of event-free survival after umbilical cord blood transplantation for Wiskott-Aldrich syndrome according to clinical score.

Figure 3. Probability of overall survival after umbilical cord blood transplantation for Wiskott-Aldrich syndrome according to year.


100
80
60
40
20
0
Probability of survival, %
less than 2 years, n=60
more than 2 years, n=30
P=0.02
0
2
4
6
8
10
12
14
16
18
20
Time after UCBT, years


clinical score 2, n=18
clinical score 4, n=18
clinical score 5, n=19
clinical score 3, n=24
Probability of survival, %
Time after UCBT, years
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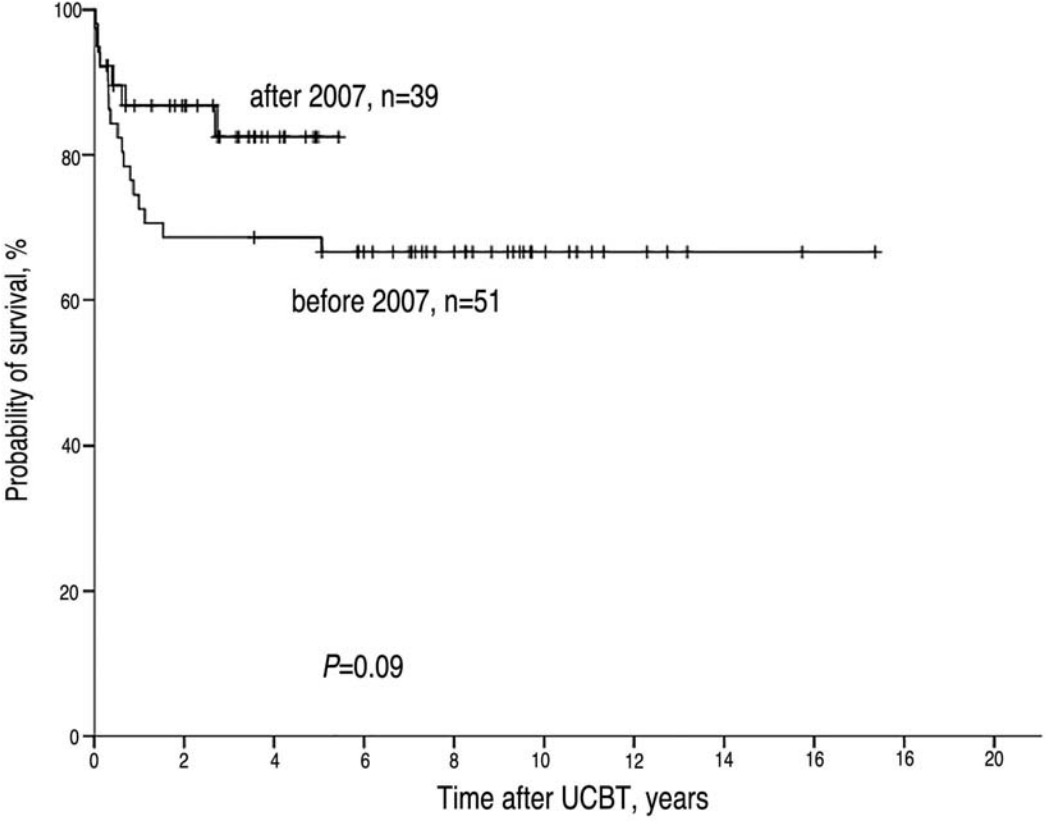
80
60
40
20
0
0
2
4
6
8
10
12
14
16
18
20


100
80
60
40
20
0
Probability of survival, %
after 2007, n=39
before 2007, n=51
P=0.09
0
2
4
6
8
10
12
14
16
16
20
Time after UCBT, years

Supplemental table

Table 1. WAS scoring system to define clinical phenotypes

	XLT				WAS	
Score	0.5	1	2	3	4	5
Thrombocytopenia	-/+	+	+	+	+	+
Small platelets	-	+	+	+	+	+
Eczema	-	-	(+)	+	++	(+)/+/++
Immunodeficiency	-	-/(+)	(+)	+	+	(+)/+
Infections	-	-	(+)	+	+/++	(+)/+/++
Autoimmunity and/or malignancy	-	-	-	-	-	+

WAS indicates Wiskott-Aldrich syndrome; XLT, X-linked thrombocytopenia.

Scoring system: -/(+), absent or mild; -/+, intermittent thrombocytopenia; (+), mild transient eczema or mild infrequent infections not resulting in sequelae; +, thrombocytopenia, persistent but therapy-responsive eczema, and recurrent infections requiring antibiotics and often IVIG prophylaxis; ++, eczema that is difficult to control and severe, life-threatening infections. Because patients with XLT may develop autoimmune disorders or lymphoma (albeit at a lower rate than those with classic WAS), a progression from a score of 1 or full donor to a score of 5 is possible for XLT.

Table 2. Median and range of absolute number (x10^9/L) of CD4+, CD8+ and CD19+ at first reported test confirmed IR, compared to median and percentiles (5th to 95th percentiles) of absolute numbers (x10^9/L) of normal values of CD4+, CD8+ and CD19+; number of patients survived, achieved engraftment and IR by age groups[1].

	5-9 mo.		9-15 mo.		15-24 mo.		2-5 yr.		5-10 yr.		10-18 yr.	
	Absolute number (x10^9/L)	***Healthy children***	Median (range)	***Healthy children***	Median (range)	***Healthy children***	Median (range)	***Healthy children***	Median (range)	***Healthy children***	Median (range)	***Healthy children***
CD4+	4.3	*2.8 (1.4-5.1)*	2.02 (1.9-2.1)	*2.3 (1.4-4.6)*	1.35 (0.7-2.6)	*2.2 (0.9-5.5)*	1.33 (0.09-3.9)	*1.3 (0.5-2.4)*	0.6 (0.28-2.7)	*1.0 (0.3-2.0)*	0.8 (0.3-1.2)	*0.8 (0.4-2.1)*
CD8+	1.75	*1.1 (0.6-2.2)*	0.8 (0.6-5.2)	*1.1 (0.4-2.1)*	0.4 (0.17-1.4)	*1.2 (0.4-2.3)*	0.6 (0.04-6.2)	*0.8 (0.3-1.6)*	1.05 (0.1-1.2)	*0.8 (0.3-1.8)*	0.36 (0.02-1.5)	*0.4 (0.2-12)*
CD19+	1.49	*1.3 (0.7-2.5)*	1.41 (0.3-1.4)	*1.4 (0.6-2.7)*	1.4 (0.89-2.7)	*1.3 (0.6-3.1)*	1.09 (0.001-2.2)	*0.8 (0.2-2.1)*	0.95 (0.5-2.1)	*0.5 (0.2-1.6)*	0.79 (0.03-1.7)	*0.3 (0.2-0.6)*

References

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